

**WHAT IS CLAIMED IS:**

1    1. A method of determining total urokinase concentration in a sample containing either  
2           or both active and inactive forms of urokinase, comprising:

3                 obtaining a first peptide corresponding to a sequence in Seq. ID No. 16  
4                 between amino acid residues 1 and 135;

5                 obtaining a second peptide corresponding to a sequence in Seq. ID No. 16  
6                 between amino acid residues 159 and 411;

7                 obtaining a third peptide corresponding to a sequence in Seq. ID No. 16  
8                 which includes amino acid residues 158 and 159;

9                 obtaining an immunological composition directed against each of said  
10              peptides;

11                 contacting aliquots of said sample with each of said immunological  
12              compositions; and,

13                 determining the quantity of each of said immunological compositions which  
14              is bound to at least one of said forms of urokinase in each of said aliquots.

1    2. The method of claim 1 wherein said first peptide is one as in any of Seq. ID Nos. 1-  
2              6.

- 1      3.     The method of claim 1 wherein said second peptide is one as in any of Seq. ID Nos.  
2                7-12.
  
  
- 1      4.     The method of claim 1 wherein said third peptide is one as in any of Seq. ID No. 13-  
2                14.
  
  
- 1      5.     The method of claim 1 wherein each of said immunological compositions has a  
2                binding affinity constant for said peptide from which it is derived that is  
3                substantially higher than its binding affinity constant for a non-urokinase protein as  
4                similar in amino acid sequence to urokinase as is trypsin.
  
  
- 1      6.     The method of claim 1 wherein said third peptide is one as in Seq. ID No. 14, and is  
2                used to derive an immunological composition which exhibits a binding affinity  
3                constant for urokinase zymogen of at least  $1 \times 10^8 \text{ M}^{-1}$ , and a binding affinity  
4                constant for forms of urokinase lacking a peptide bond between amino acid residues  
5                158 and 159 of Seq. ID No. 16 of at least approximately 10-fold lower than that for  
6                urokinase zymogen.
  
  
- 1      7.     The method of claim 1 wherein an additional peptide corresponding to a sequence in  
2                Seq. ID No. 16 which includes amino acid residues 135 and 136 is obtained and  
3                used to derive said immunological composition.

- 1      8.      The method of claim 7 wherein said additional peptide is that of Seq. ID No. 17.
  
- 1      9.      The method of claim 1 wherein the immunological composition is an antiserum, an  
2                  antibody, a hybridoma, or a supernatant of a hybridoma, obtained via injection into a  
3                  mammal of said peptide.
  
- 1      10.     The method of claim 1 wherein said determination of said quantity of each of said  
2                  immunological compositions is carried out by radiolabeling said sample or said  
3                  immunological composition.
  
- 1      11.     The method of claim 1 wherein said determination of said quantity of each of said  
2                  immunological compositions is carried out by:
  - 3                  determining the amount of low molecular weight urokinase in said sample  
4                  which binds to one or more of said immunological compositions directed against  
5                  said peptide corresponding to a sequence in Seq. ID No. 16 between amino acid  
6                  residues 159 and 411, but which does not bind to one or more of said  
7                  immunological compositions directed against said peptide corresponding to a  
8                  sequence in Seq. ID No. 16 between amino acid residues 1 and 135;
  
  - 9                  determining the amount of high molecular weight urokinase in said sample

10 which binds to one or more of said immunological compositions directed against  
11 said peptide corresponding to a sequence in Seq. ID No. 16 between amino acid  
12 residues 1 and 135 or between amino acid residues 159 and 411, but which does not  
13 bind to one or more of said immunological compositions directed against said  
14 peptide corresponding to a sequence in Seq. ID No. 16 which includes amino acid  
15 residues 158 and 159; and,

16 determining the amount of urokinase zymogen in said sample which binds  
17 to one or more of said immunological compositions directed against said peptide  
18 corresponding to a sequence in Seq. ID No. 16 which includes amino acid residues  
19 158 and 159.

1 12. The method of claim 1 wherein said determination of said quantity of each of said  
2 immunological compositions is carried out by:

3 determining the amount of low molecular weight urokinase in said sample  
4 which binds to one or more of said immunological compositions directed against  
5 said peptide corresponding to a sequence in Seq. ID No. 16 between amino acid  
6 residues 159 and 411, but which does not bind to one or more of said  
7 immunological compositions directed against a peptide corresponding to Seq. ID  
8 No. 17;

9 determining the amount of high molecular weight urokinase in said sample  
10 which binds to one or more of said immunological compositions directed against  
11 said peptide corresponding to a sequence in Seq. ID No. 16 between amino acid  
12 residues 1 and 135 or between amino acid residues 159 and 411, but which does not

13 bind to one or more of said immunological compositions directed against said  
14 peptide corresponding to a sequence in Seq. ID No. 16 which includes amino acid  
15 residues 158 and 159; and,

16 determining the amount of urokinase zymogen in said sample which binds  
17 to one or more of said immunological compositions directed against said peptide  
18 corresponding to a sequence in Seq. ID No. 16 which includes amino acid residues  
19 158 and 159.

1 13. A method of determining total urokinase concentration in a sample containing either  
2 or both active and inactive forms of urokinase, comprising:

3 obtaining a peptide as in any of Seq. ID Nos. 1-6;

4 obtaining a peptide as in any of Seq. ID Nos. 7-12;

5 obtaining a peptide as in any of Seq. ID No. 13-14;

6 obtaining an immunological composition selected from the group of  
7 immunological compositions consisting of an antisera, an antibody, a hybridoma, or  
8 a supernatant of a hybridoma, said immunological compositions each obtained via  
9 injection into a mammal of said peptide;

10 contacting aliquots of said sample with each of said immunological  
11 compositions; and,

12           determining the quantity of each of said immunological compositions which  
13        is bound to at least one of said forms of urokinase in each of said aliquots, wherein  
14        said determination of the quantity of each of said immunological compositions is  
15        carried out by:

16           determining the amount of low molecular weight urokinase in said sample  
17        which binds to one or more of said immunological compositions directed against  
18        said peptide corresponding to a sequence in Seq. ID No. 16 between amino acid  
19        residues 159 and 411, but which does not bind to one or more of said  
20        immunological compositions directed against said peptide corresponding to a  
21        sequence in Seq. ID No. 16 between amino acid residues 1 and 135;

22           determining the amount of high molecular weight urokinase in said sample  
23        which binds to one or more of said immunological compositions directed against  
24        said peptide corresponding to a sequence in Seq. ID No. 16 between amino acid  
25        residues 1 and 135 or between amino acid residues 159 and 411, but which does not  
26        bind to one or more of said immunological compositions directed against said  
27        peptide corresponding to a sequence in Seq. ID No. 16 which includes amino acid  
28        residues 158 and 159; and,

29           determining the amount of urokinase zymogen in said sample which binds  
30        to one or more of said immunological compositions directed against said peptide  
31        corresponding to a sequence in Seq. ID No. 16 which includes amino acid residues  
32        158 and 159.

1     14.   A kit for determining total urokinase concentration in a sample containing either or

2 both active and inactive forms of urokinase, comprising:

3                 immunological compositions, selected from the group of immunological  
4                 compositions consisting of an antisera, an antibody, a hybridoma, or a supernatant  
5                 of a hybridoma;

6                 said immunological compositions each obtained via injection into a mammal  
7                 of a peptide directed against each of a set of peptides including: a first peptide  
8                 corresponding to a sequence in Seq. ID No. 16 between amino acid residues 1 and  
9                 135, a second peptide corresponding to a sequence in Seq. ID No. 16 between  
10                amino acid residues 159 and 411, and a third peptide corresponding to a sequence in  
11                Seq. ID No. 16 which includes amino acid residues 158 and 159; and,

12                instructions for contacting aliquots of said sample with each of said  
13                immunological compositions, and determining the quantity of each of said  
14                immunological compositions which is bound to at least one of said forms of  
15                urokinase in each of said aliquots.

1       15. The kit of claim 14 wherein said first peptide is one as in any of Seq. ID Nos. 1-6.

1       16. The kit of claim 14 wherein said second peptide is one as in any of Seq. ID Nos. 7-  
2                12.

1       17. The kit of claim 14 wherein said third peptide is one as in any of Seq. ID No. 13-14.



10           determine the amount of low molecular weight urokinase in said sample  
11          which binds to one or more of said immunological compositions directed against  
12          said peptide corresponding to a sequence in Seq. ID No. 16 between amino acid  
13          residues 159 and 411, but which does not bind to one or more of said  
14          immunological compositions directed against said peptide corresponding to a  
15          sequence in Seq. ID No. 16 between amino acid residues 1 and 135;

16           determine the amount of high molecular weight urokinase in said sample  
17          which binds to one or more of said immunological compositions directed against  
18          said peptide corresponding to a sequence in Seq. ID No. 16 between amino acid  
19          residues 1 and 135 or between amino acid residues 159 and 411, but which does not  
20          bind to one or more of said immunological compositions directed against said  
21          peptide corresponding to a sequence in Seq. ID No. 16 which includes amino acid  
22          residues 158 and 159; and,

23           determine the amount of urokinase zymogen in said sample which binds to  
24          one or more of said immunological compositions directed against said peptide  
25          corresponding to a sequence in Seq. ID No. 16 which includes amino acid residues  
26          158 and 159.

- 1       22.     A peptide for determining total urokinase concentration in a sample as in any of  
2       Seq. ID Nos. 1-15 and 17.
  
- 1       23.     An antisera obtained via injection into a mammal of a peptide for determining total

2           urokinase concentration in a sample as in any of Seq. ID Nos. 1-15 and 17.

1   24.   An antibody obtained via injection into a mammal of a peptide for determining total  
2       urokinase concentration in a sample as in any of Seq. ID Nos. 1-15 and 17.

1   25.   A hybridoma producing an antibody obtained via injection into a mammal of a  
2       peptide for determining total urokinase concentration in a sample as in any of Seq.  
3       ID Nos. 1-15 and 17.

1   26.   A supernatant of a hybridoma producing an antibody obtained via injection into a  
2       mammal of a peptide for determining total urokinase concentration in a sample as in  
3       any of Seq. ID Nos. 1-15 and 17.